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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application : Mark T. Spitler et al
 Application No. : 09/350,466
 Filed : July 9, 1999
 For : DYE DESORPTION MOLECULAR INDICATOR
 Examiner : L. Cross
 Attorney's Docket : CHEMM-101XX

Group Art Unit: 1743

 I hereby certify that this correspondence is being deposited
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DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
 Washington, D.C. 20231

Sir:

I, Louis S. Stuhl, citizen of The United States, residing at
 7 Orchard Road, Bedford, Ma 01730, hereby declare as
 follows:

1. I am an inventor of the invention described and claimed
 in the above mentioned patent application.

2. I have received training in inorganic and organometallic chemistry having received the M. S. degree in inorganic chemistry and the Ph. D. degree in organometallic chemistry from Cornell University.

3. I have had work experience in inorganic and organometallic chemistry at the University of California, Berkeley as a Postdoctoral Fellow from 1978 through 1979 and at Brandeis University as an Assistant Professor from 1979 to 1986. Since then, I have acquired additional work experience in these areas as well as organic chemistry, colloid chemistry and coating science at American Optical Corp. as a staff Chemist from 1987 through 1988, at Polaroid Corp. as a Senior Scientist from 1988 through 1996, and at ChemMotif, Inc. as Vice-President and Senior Research Fellow from 1997 to the present. I have been the author or co-author of approximately 18 technical articles, one patent and several delivered papers relating to inorganic chemistry, organometallic chemistry, physical chemistry, and dye sensitized solar cells.

4. I understand that the Examiner for U.S. Patent Application Serial No. 09/350,466 has rejected claims of this application in part because (a) it is believed that the term "high boiling plasticizer/solvent" is unclear, (b) it

is believed that the single layer device is significantly different from the multi-layer device, and (c) it is believed that the instant invention is obvious over Clement and Burleigh.

5. In the application, on pages 6, 13 and elsewhere, there is description of the "high boiling plasticizer/solvent". In addition, as discussed in the Amendment being filed herewith, the "high boiling plasticizer/solvent" is not the same as a traditional plasticizer. Rather the term is defined to be a solvent, which remains in the device for a long period of time and performs the role of a solvent, moving the radiant energy-detectable material to the appropriate location in the device. If the material evaporates from the device, it can be replaced at any time and still allow the device to perform its function.

We have conducted an experiment to demonstrate that the "plasticizer/solvent" performs as described above (i.e. as a solvent).

Experiment 1

Objective: To determine whether the high boiling plasticizer functions as a solvent, not as a traditional plasticizer.

If the coating is exposed to an elevated temperature so as to evaporate a significant amount of the plasticizing solvent, the performance of the analytical detection element is seriously impaired or even completely eliminated. This shows that the plasticizer/solvent is necessary for device functioning. The coating remains intact after this plasticizer/solvent loss, showing that the plasticizing effect of the plasticizer/solvent is secondary and in some formulations completely unnecessary.

The effects of the heat-induced plasticizer/solvent loss are completely reversed by overcoating the analytical element with a thin layer of pure plasticizer/solvent that then spontaneously soaks into the element and restores its function.

A. A multilayer coating similar to Example 1 in the application was prepared with the substitution of a 30/70 mixture of 1,2,6-hexanetriol/1,5-pentanediol for 1,4-butanediol. The cyan response of the coating to ethylbenzene was recorded to give the baseline response (see results below at A).

B. A sample of the multi-layer coating was then heated to 80 C for 24 hours to evaporate the plasticizing solvent and its

response to ethylbenzene vapor was measured. The results from this sample without plasticizing solvent is shown at B below.

C. Samples of the coating after heating were then overcoated with 1,2,6-hexanetriol/1,5-pentanediol using a #12 Mayer rod (i.e., the plasticizer solvent was restored). They were then exposed to ethylbenzene and reread (see data at C below).

Stage		Change in cyan OD after 90 minutes
A.	Original coating	0.50
B.	Coating with plasticizer/solvent removed	0.00
C.	Coating with 1,2,6- hexanetriol/1,5-pentanediol restored	0.45

Note that the ability to sense ethylbenzene is completely lost (zero optical density change on exposure) after extended heat treatment. No cracking or other physical deterioration of the coating occurred as a result of the heat treatment (i.e. we would expect these defects if the material were performing as a traditional plasticizer). Adding an approximately 27 micrometer thick coating of the

plasticizer/solvent mixture to the dried-out coating restored 90% of its sensing function. Thus, the plasticizer/solvent can be seen to function as a solvent enabling analyte adsorption and dye desorption in the dye desorption sensor element.

6. We have conducted an experiment to show that the single- and multiple-layer devices perform in a similar manner. Analyte induced dye-desorption takes place in the single-layer device via the same mechanism as it takes place in the layer in the multi-layer system that contains the adsorbent solid with dye adsorbed onto it. This shows that the single-layer and multi-layer systems function via the same reaction mechanism with analytes (i.e., analyte-induced dye desorption), and that these coatings are equivalent except in regards to how the analytical signal is read from the analytical element.

The multi-layer coating structure is necessary to remove the desorbed dye from the vicinity of the adsorbent so that it can be seen by the naked eye without interference from the adsorbent's color. However, the actual sensing function (i.e. reaction with analyte) is carried out entirely in the first region (where the initial reaction takes place), which contains the adsorbent with the preadsorbed dye. A single-

layer sensor can be made to function by providing an instrumental readout that does not require removal of the desorbed dye in order for its desorption to be detected. This is shown by the following experiment:

Experiment 2

Objective: to show that the analyte sensing mechanism of a single-layer sensor chemically operates in a manner identical to that of a multi-layer sensor.

A multilayer coating was assembled after the manner of Example 1 in the application, using 1,4-butanediol in place of 2-methyl-1,3-propanediol. The response of this coating to toluene vapor was measured for twenty minutes after the manner of Example 6 of the patent application. Response was judged by visual examination and by measurement with an X-Rite 310 color densitometer. This instrument records the light reflected off of an object and analyzes it in terms of a gray-scale simulated visual response as well as separating it into its component reflective primary colors: cyan, magenta, and yellow.

A single layer coating was made comprised of only the carbon-containing layer of the above element on a clear polymer base. It was assembled in a test system as in

Example 1 of the Application, but with the vessel inserted into a commercial spectrofluorimeter so that fluorescence from within the carbon layer could be measured. The spectrofluorimeter records the fluorescence signal as photons counted on an arbitrary scale. A strong response is one that is several powers of ten greater than the background count. The layer was exposed to toluene and the signal recorded after twenty minutes.

	response before toluene vapor exposure	response after 20 min. toluene vapor exposure
Multilayer coating: colorimetric response	white with faint pink cast	strong magenta color: optical density increase in magenta component of >1.0
Single layer coating: fluorescence measurement	no detectable fluorescence	strong fluorescence signal from spectrofluorimeter: ~80,000 counts at a signal:noise ratio of $>10^5$

The single-layer coating is not fundamentally different from the multi-layer one; it is simply a subcomponent of the multi-layer analytical element. The additional layers in the

multi-layer coating enable visual readout and remove the need for an instrumental readout. This single-layer system takes advantage of the well-known fact that fluorescent dyes have their fluorescent emission quenched when they are adsorbed onto many solids, especially colored ones. Thus, no fluorescence is observed from a normally fluorescent dye such as Pyronin Y when all of the dye is adsorbed onto activated carbon. The dye that is desorbed as a result of exposure to analyte immediately becomes fluorescent, and this fluorescence can be detected even in the presence of the dark carbon through the use of a sufficiently sensitive photon detector such as is found in commercial spectrofluorimeters. The first layer of a multi-layer device thus functions the same as the one layer of the single layer device in its mechanism of capturing analyte which then displaces dye.

7. I understand that the Examiner believes that the instant invention is obvious over Clement combined with Burleigh. As indicated in the application (page 1, line 20ff) and in the Amendment, the device is intended to be a broad screen analyzer, as opposed to the specific analyzers currently available and described in Clement. That vapor-induced dye desorption from a solid can be used as a chemical sensing mechanism is novel and unexpected. Neither Clement nor

Burleigh contemplates desorption of a dye from an adsorbent in direct response to the presence of an analyte; in fact, desorption would defeat the purpose of Burleigh's method, and the possibility of intentional desorption is not contemplated therein.

We theorized that dyes with heats of adsorption in a range appropriate for competitive binding with common organic analytes should exist. This is contrary to common thinking, since most commercial dyes are designed to be strongly adsorbed. For example, it is unexpected that suitable dyes could be found to function in an activated carbon-containing sensor because activated carbon is commonly used to irreversibly remove colored compounds from solutions, including those in organic solvents. This is a common procedure in undergraduate organic chemistry laboratories, as described in e.g. K. L. Williamson "Macroscale and Microscale Organic Experiments," 2nd Ed., D. C. Heath & Co., Lexington, MA 1994, p. 44-45. DARCO G-60 is well-known in the art for use for this purpose. That certain dyes can be usefully desorbed from activated carbon is thus not obvious and is of unexpected benefit in providing a broad screen sensor element.

We have conducted experiments to show that the specific combination of dye and adsorbent support materials that allow the invention to work are quite limited and that it is an unexpected benefit that they work.

Experiment 3

Objective: to determine whether there exist combinations of dye, adsorbent and plasticizer/solvent that enable analysis via competitive adsorption/desorption.

This experiment demonstrates that only selected combinations of dyes with carbon are suitable for use in this multilayer analytical element. Whether dyes suitable for use with e.g. DARCO G-60 carbon exist was determined via the following test. (results are summarized in the table below)

Test 1. Samples of DARCO in water were prepared by adding 50 mg of DARCO to 5.0 ml of water in a test tube. A 0.75 ml aqueous aliquot containing 1.1 micromoles of a dye was added to one of these DARCO samples and the tube shaken. A suitable dye will adsorb onto the carbon resulting in a clear solution; an unsuitable dye will not adsorb.

Test 2. The supernatant in the test tube was decanted and 3 ml of a plasticizing solvent such as 1,4-butanediol or

ethylene glycol were added. A suitable dye will not desorb on the addition of the plasticizing solvent.

Test 3. Sensitivity to toluene or other analytes was tested through the addition of 50 microliters of toluene or other analyte to the 3ml solution containing dye on DARCO carbon. If the dye desorbs in the presence of analyte, producing a colored solution, it is suitable for use in the multilayer coating. If no dye desorbs, and the solution remains clear, the dye is unsuitable.

Dye (tested with toluene analyte)	Test 1	Test 2	Test 3	Result
Rose Bengal	colored solution			dye unsuitable
Alizarin Red S	clear solution	clear solution	clear solution	dye unsuitable
Pyronin Y	clear solution	clear solution	colored solution	suitable

Other commercial dyes that have proven unsuitable include common azo dyes such as Amaranth and Acid Violet 7, triphenylmethane dyes such as Crystal Violet and Brilliant Green, and indigoid dyes such as Indigo Carmine.

In our initial search for the hypothesized suitable dyes, we found that, of a selection of dyes biased towards cationic dyes that were of interest to us for unrelated reasons, only 1/3 of the dyes passed the above initial screening with propylene glycol, and only 1/5 passed in an alternate plasticizer/solvent propylene carbonate. (See Table I, page 9 of the Application, where a system using propylene carbonate as the plasticizer/solvent was discussed.) The bias towards cationic dyes has proven fortuitous, as we have had no success with the more common and less expensive anionic and neutral dye classes in our currently preferred plasticizer/solvents. Additional tests have shown that only a minority of commercial cationic dyes are suitable, and that different dyes are suitable for different adsorbent and plasticizer/solvent combinations. For diols or triols as plasticizer/solvents and Darco G-60, the success rate is highest with tricyclic cationic dyes, and with this discovery, we are able to fine tune our dye selection process. Thus we have shown that very few dyes are acceptable for usage in our device and thus that the results

with these dyes provide an unexpected benefit. The results found here would not have been anticipated from Clement and Burleigh.

8. I declare further that all statements made herein to my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Nov. 5, 2001
Date

Louis S. Stuhl
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Macroscale and Microscale Organic Experiments

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ually, keeping it at the boil, until all the solute dissolves. Addition of a boiling stick or a boiling chip to the solution once most of the solid is gone will promote even boiling. It is not difficult to superheat the solution, that is, heat it above the boiling point with no boiling taking place. Once the solution does boil, it does so with explosive violence. Never add a boiling chip or boiling stick to a hot solution. A glass rod with a flattened end can sometimes be of use in crushing large particles of solute to speed up the dissolving process. Be sure no flames are nearby when working with flammable solvents.

Be careful not to add too much solvent. Note how rapidly most of the material dissolves and then stop adding solvent when you suspect that almost all the desired material has dissolved. It is best to err on the side of too little solvent rather than too much. Undissolved material noted at this point could be an insoluble impurity which never will dissolve. Allow the solvent to boil, and if no further material dissolves, proceed to step 4 to remove suspended solids from the solution by filtration, or if the solution is colored, go to step 3 to carry out the decolorization process. If the solution is clear, proceed to step 5, crystallizing the solute.




MICROSCALE
AND MACROSCALE

3. Decolorizing the Solution; Use of Pelletized Norit

The vast majority of pure organic chemicals are colorless or a light shade of yellow. Occasionally, a chemical reaction will produce high-molecular-weight by-products that are highly colored. The impurities can be adsorbed onto the surface of activated charcoal simply by boiling the solution with charcoal. Activated charcoal has an extremely large surface area per gram (several hundred square meters) and can bind a large number of molecules to this surface. On a commercial scale, the impurities in brown sugar are adsorbed onto charcoal in the process of refining sugar.

In the past, laboratory manuals have advocated the use of finely powdered activated charcoal for removal of colored impurities. This has two drawbacks. Because the charcoal is so finely divided, it can only be separated from the solution by filtration through paper, and even then some of the finer particles pass through the filter paper. And the presence of the charcoal completely obscures the color of the solution so that adding the correct amount of charcoal is mostly a matter of luck. If too little charcoal is added, the solution will still be colored after filtration, making repetition necessary; if too much is added, it will absorb some of the product in addition to the impurities. We have found that charcoal extruded as short cylindrical pieces measuring about 0.8×3 mm made by the Norit Company solves both these problems. It works just as well as the finely divided powder, and it does not obscure the color of the solution. It can be added in small portions until the solution is decolorized, and the size of the pieces makes it easy to remove from the solution.¹



*Pelletized Norit (Also called
active carbon, activated
charcoal, decolorizing carbon)*

1. Available from Aldrich Chemical Co., 940 West St. Paul Ave., Milwaukee, WI 53233, catalog number 32942-8 as Norit RO 0.8. This form of Norit is an extrudate 0.8 mm dia. It has a surface area of 1000 m²/g and a total pore volume of 1.1 mL/g.

On both a microscale and a macroscale, simply add a small amount (0.1% of the solute weight is sufficient) of pelletized Norit to the colored solution and then boil the solution for a few minutes. Be careful not to add the charcoal pieces to a superheated solution; the charcoal functions like hundreds of boiling chips and will cause the solution to boil over. Remove the Norit by filtration as described in step 4.

4. Filtering Suspended Solids

The filtration of a solution to remove solid impurities or charcoal can be done in a number of ways. Processes include gravity filtration, pressure filtration, decantation, or removal of the solvent using a Pasteur pipette. Vacuum filtration is not used because the hot solvent will cool during the process and the product will crystallize in the filter.

Microscale Procedure

(A) Removal of Solution with a Pasteur Pipette. If the solid impurities are large in size, they can be removed by filtration of the liquid through the small space between the square end of a Pasteur pipette and the bottom of a reaction tube (Fig. 3.3). Expel air from the pipette as it is being pushed to the bottom of the tube. Use a small additional quantity of solvent to rinse the tube and pipette. Anhydrous calcium chloride, a drying agent, is removed easily in this way. Removal of very fine material, such as traces of charcoal, is facilitated by filtration of the solution through a small piece of filter paper (3 mm²) placed in the reaction tube. This process is even easier if the filter paper is the thick variety, such as that from which Soxhlet extraction thimbles are made.²

(B) Filtration in a Pasteur Pipette. To filter 0.1 to 2 mL of a solution, dilute the solution with enough solvent that the solute will not crystallize out at room temperature. Prepare a filter pipette by pushing a tiny bit of cotton into a Pasteur pipette, put the solution to be filtered into this filter pipette using another Pasteur pipette, and then force the liquid through the filter using air pressure from a pipette bulb (Fig. 3.4). Fresh solvent should be added to rinse the pipette and cotton. The filtered solution is then concentrated by evaporation. One problem encountered with this method is using too much cotton packed too tightly in the pipette so that the solution cannot be forced through it. To remove very fine impurities, such as traces of decolorizing charcoal, a 3- to 4-mm layer of Celite filter aid can be added to the top of the cotton.

(C) Pressure Filtration with Micro Büchner Funnel. The technique applicable to volumes from 0.1 to 5 mL is the use of the *micro Büchner funnel*. It is made of polyethylene and is fitted with a porous *polyethylene frit* 6 mm in

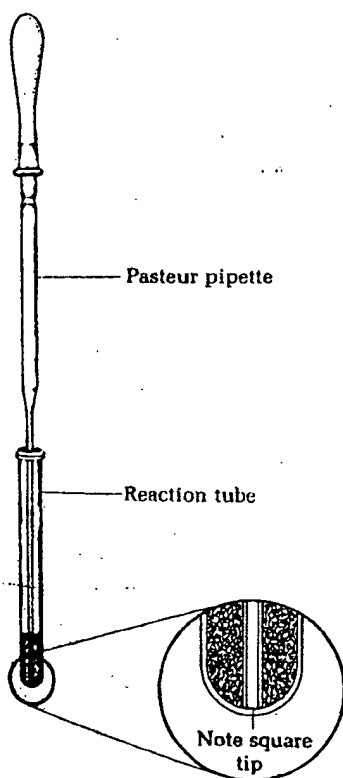


FIG. 3.3 Filtration using the Pasteur pipette and reaction tube.

2. J. L. Belletire and N. O. Mahmoodi, *J. Chem. Ed.* 66:964, 1989.